SPORULATION OF *Amblyospora* (MICROSPORA) IN FEMALE *Culex salinarius*: INDUCTION BY 20-HYDROXYECDYSONE

BY

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TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	iv
LIST OF FIGURES	v
ABSTRACT	vi
INTRODUCTION	1
LITERATURE REVIEW	3
Introduction	3
Regulation of Ovarian Development in Mosquitoes	4
Exploitation of Host Hormones by Parasites	13
The Biology of Dimorphic Microsporidia	22
EXPERIMENTAL DETERMINATION OF THE INDUCER OF SPORULATION OF *Amblyospora*	31
Introduction	31
Materials and Methods	32
Results	40
Discussion	56
LITERATURE CITED	63
BIOGRAPHICAL SKETCH	73

LIST OF TABLES

Table		Page
1.	EFFECT OF DECAPITATION OF NEWLY BLOOD-FED *C. salinarius* ON SPORULATION OF *Amblyospora* SP.	41
2.	EFFECT ON SPORULATION OF *Amblyospora* SP. OF INJECTION OF HOST EGG MACERATE INTO *C. salinarius*	43
3.	EFFECT OF SPORULATION OF *Amblyospora* SP. OF ORGAN IMPLANTATION INTO *C. salinarius*	44
4.	EFFECT ON SPORULATION OF *Amblyospora* SP. OF TOPICAL APPLICATION OF JH I AND METHOPRENE TO *C. salinarius*	46
5.	EFFECT ON SPORULATION OF *Amblyospora* SP. OF INJECTION OF 20-OH ECDYSONE INTO *C. salinarius.*	47
6.	EFFECT ON SPORULATION OF *Amblyospora* SP. OF INJECTION OF CHOLESTEROL INTO *C. salinarius*	48
7.	EFFECT ON SPORULATION OF *Amblyospora* SP. OF OVARIECTOMY OF *C. salinarius*.	50
8.	EFFECT ON SPORULATION OF *Amblyospora* SP. OF INJECTION OF CATECHOLAMINES INTO *C. salinarius.*	51

LIST OF FIGURES

Figure		Page
1.	BLOOD-FED AND DECAPITATED *C. salinarius*.	34
2.	ABDOMEN OF *C. salinarius* WITH AN OVARY BEING REMOVED . . .	35
3.	CAGES FOR POSTOPERATIVE *C. salinarius*.	37
4.	PHOTOMICROGRAPHS OF *Amblyospora* SP. STAINED WITH GIEMSA STAIN. A. SPORES. B. VEGETATIVE STAGES	38
5.	ELECTRON MICROGRAPHS OF IMMATURE SPORES OF *Amblyospora* SP. A. 20-OH ECDYSONE INDUCED. B. BLOOD MEAL INDUCED.	53
6.	AUTORADIOGRAPH OF *C. salinarius* OOCYTES SHOWING SILVER GRAINS IN DEVELOPING CHORION	55
7.	AUTORADIOGRAPH OF *Amblyospora* SP. SPORES AND *C. salinarius* TRACHEAE	57
8.	PROPOSED SCHEME FOR INDUCTION OF SPORULATION OF *Amblyospora* SP. IN *C. salinarius*	58

Abstract of Dissertation Presented to the Graduate Council of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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Amblyospora sp. in *Culex salinarius* is a dimorphic microsporidium in which the form found in female hosts sporulates after the host has taken a blood meal. Decapitation experiments with blood-fed infected mosquitoes indicate that sporulation is not a response to nutritional factors. Topical treatments with juvenile hormone I and methoprene; injections of egg macerate, dopamine, and n-acetyldopamine; and implantation of activated brains, ovaries, and cockroach corpora cardiaca did not induce sporulation. Injection of 100 ng of 20-hydroxyecdysone induced sporulation, but injection of cholesterol did not. Since mosquito ovaries secrete ecdysteroids in response to a blood meal, infected *C. salinarius* were ovariectomized and then either

fed blood or injected with 20-hydroxyecdysone. Spores were found in those insects injected with the hormone, but not in those given blood. These results suggest that 20-hydroxyecdysone or some substance that appears in the mosquito hemolymph as a result of the hormone's presence is the cue to which Amblyospora responds by forming spores. Sequential examination of blood-fed infected mosquitoes showed that sporulation followed ecdysteroid secretion by an interval consistent with a causal relationship.

In insects, 20-hydroxyecdysone plays an important role in sclerotization by inducing dopa decarboxylase synthesis. The possibility that sclerotin-like proteins are present in the spore wall of Amblyospora was investigated by use of cytochemical tests for phenol oxidases and catecholamines, autoradiography with ^{3}H-dopamine, and injection of dopa decarboxylase inhibitors. The results indicate that sclerotins are absent in the spore wall. Therefore, it appears that the action of 20-hydroxyecdysone on Amblyospora is unrelated to dopa-decarboxylase.

INTRODUCTION

Protozoans of the phylum Microspora are obligate intracellular parasites of extremely broad host range, but great specialization of life style. They are characterized by a unicellular spore stage with a uninucleate or binucleate sporoplasm and a simple or complex extrusion apparatus always with a polar filament and polar cap (Levine et al., 1980). Weiser (1976) believes that there may be at least one microsporidian species in every living invertebrate species in addition to the large number found in vertebrates. They appear to be particularly abundant as parasites of the Arthropoda.

The importance of microsporidia in regulation of arthropod population is well documented (Henry, 1981). This regulation may be an entirely natural phenomenon, as in the case of Nosema fumiferana and its host Choristoneura fumiferana (Wilson, 1977), or it may be the result of human intervention for pest control, as in the case of Nosema locustae and a number of host grasshopper species (Henry, 1971).

In mosquitoes microsporidia are among the most common and widespread pathogens. Hazard and Chapman (1977) list over 100 mosquito hosts of these parasites, and Chapman (1974) suggests that all mosquito species have microsporidia associated with them.

In spite of the importance of microsporidia, knowledge of their biology remains rudimentary. This study was undertaken to elucidate certain aspects of the basic biology of an undescribed species of Amblyospora that parasitizes Culex salinarius, a common mosquito pest throughout most of the U.S. and Canada east of the Rocky Mountains.

This dissertation includes a literature review and details experiments on the control of sporulation of Amblyospora species in adult female Culex salinarius. The literature review covers three subjects: ovarian development in mosquitoes, the effects of host hormones on symbiotes, and the biology of dimorphic microsporidia. The experiments on sporulation of Amblyospora determined that this process, which occurs after the host feeds on blood, is not a response to nutrition factors but is hormone related. Specifically, sporulation was induced by injection of 20-hydroxyecdysone (20-OH ecdysone). This suggested a possible role of dopa-decarboxylase in spore formation. Experiments conducted to detect such a role yielded negative results.

LITERATURE REVIEW

This literature review is comprised of three parts of manifestly disparate subject matter. Each topic, however, is of fundamental importance to the research presented in this thesis. The first part deals with the reproductive physiology of female mosquitoes. This is a matter central to the study because it is clearly an alteration in the physiological state of the host insects at the time of egg development that is the stimulus for sporulation in Amblyospora. The effects of host hormones on symbiotes is dealt with in the second section. The final section reviews the biology of dimorphic microsporidia, a subject of growing importance as more species are discovered and their practical utility is demonstrated.

Regulation of Ovarian Development in Mosquitoes

The literature on mosquito ovarian development has been reviewed by Clements (1963), by Laurence (1977), and by Fuchs and Kang (1981). The following review will consider anatomical development only briefly and concentrate on hormonal regulation of oogenesis. It should be noted that the information presented here was obtained primarily from experiments with Aedes aegypti, but the generalizations appear to hold for other mosquitoes (Laurence, 1977).

Because the concept of autogeny is important to the research on mosquito oogenesis it is described here. Most mosquitoes require blood-feeding for maturation of all the eggs they produce. Such mosquitoes are referred to as anautogenous. Autogenous mosquitoes are those which are able to produce at least their first clutches of eggs without taking a blood-meal.

Anatomical Development

The ovaries of mosquitoes are paired organs which may have as many as 300 ovarioles per pair in some species (Harwood and James, 1979). As in all Diptera, the ovary is of the polytrophic type with the follicles which are proximal to the oviduct differentiated into an oocyte and 7 nurse cells within a follicular epithelium. Penproximal and succeeding follicles are poorly differentiated (Laurence, 1977).

Follicular development of mosquitoes was divided into a series of stages by Christophers (1911, as cited in Clements, 1963), and these are still used. In Stage I there is no yolk in the oocyte. Stage II is the resting stage in which the follicles remain until the mosquito takes a blood-meal except in cases of autogeny. There are a few yolk

granules around the nucleus of the oocyte, which is readily discernible from the nurse cells. In Stage III yolk obscures the oocyte nucleus, and the oocyte occupies from 1/2 to 3/4 of the follicle. The Stage IV follicle is elongate with less than 1/10 of its volume occupied by nurse cells. In Stage V chorionic structures are visible, and the eggs become ready for oviposition.

A few hours after a female feeds on blood, the follicle cells separate allowing the oocyte full exposure to vitellogenins in the hemolymph (Roth and Porter, 1964). The oocyte surface shows micropinocytotic vesicles within 3 hours of blood feeding (Anderson and Spielman, 1971). During later stages of vitellogenesis (30-48 hr in Aedes aegypti) the pinocytotic mechanism is diminished as intercellular channels become occluded by desmosomes, and vitelline membrane plaques separate the oocyte and follicular epithelium (Anderson and Spielman, 1971).

Penproximal follicles differentiate during vitellogenic growth of proximate oocytes. The oocyte, nurse cells, and follicular epithelium are discernible in histological sections 20 hours after the blood meal (Laurence, 1977). The penproximal follicle reaches Stage II and is inhibited from further development by the presence of eggs in the proximal follicle (Meola and Lea, 1972).

Hormonal Regulation of Previtellogenic Development

Larson and Bodenstein (1959) employed various surgical techniques to arrive at the conclusion that juvenil hormone (JH) was the sole endocrine regulator of mosquito egg development. JH is secreted by the corpora allata (CA), which are located in the anterior thorax, and are

axonally connected to the brain. Lea (1963, 1969) used ablation and implantation to clarify the role of JH. If the CA were removed within 1 hr of emergence, no yolk was deposited after subsequent blood-feeding. If the CA were removed 3-5 days after emergence, egg maturation followed blood-feeding. This indicated that JH release by the CA, though it was required for development of competency to respond to a blood-meal, was not in itself sufficient for regulation of vitellogenesis. Ablation and implantation experiments with the median neurosecretory cells (MNC) of the brain showed that these too were needed for egg development, but that they regulated a different process from that controlled by the CA (Lea, 1967). Since Lea (1969) ablated the MNC after the critical period for stimulation of CA, he observed only their requirement for vitellogenesis.

Gwadz and Spielman (1973) reported evidence that JH from the CA controls differentiation of a follicle to the resting stage. They found that the proximate follicles of females that had been decapitated or allatectomized within 1 hr of emergence failed to reach the resting stage and did not accumulate yolk in response to a blood-meal. Application of JH analogues or implantation of CA restored normal development in ablated animals. When the allatectomies were performed 3 days after emergence, development of the proximate follicles proceeded normally, but those that were penproximate did not reach the resting stage after blood-feeding and did not accumulate yolk after a second blood-meal unless treated with a JH analogue. This indicated that a brain stimulus causes the CA to release JH that was needed to bring not only the first follicle to resting stage, but also subsequent follicles after ensuing blood meals. Feinsod and Spielman (1980b) confirmed the need for a

brain stimulus of CA by decapitating females 18 hr after blood-feeding. This prevented development of the penproximate follicle to Stage III.

Feinsod and Spielman (1980a) also demonstrated a nutritional requirement for previtellogenic ovarian development. By rearing mosquitoes under crowded conditions, they obtained nutrient-deprived adults with proximate follicles that did not grow to resting stage after emergence. Such follicles developed to resting stage after feeding on sucrose or blood, or after topical application of JH analogue.

Stimulation of Vitellogenesis

Anautogenous mosquitoes begin vitellogensis in response to feeding on vertebrate blood. Larsen and Bodenstein (1959) ascribed the stimulus solely to stretching of the midgut resulting from engorement on blood. A more recent report by Bellamy and Bracken (1971) presents contradictory evidence and suggests that only the nutrient content of the blood-meal is crucial. Spielman and Wong (1974) found that a partial blood-meal of insufficient volume to induce vitellogenesis will do so if supplemented by anal injection of saline, but injection of saline alone will not stimulate vitellogenesis. They also found that feeding on the cellular fractions of blood and globulin fractions and albumin fractions of serum all stimulate oogenesis, but feeding on hemoglobin does not. It appears that oogenesis depends on distention of the midgut as well as on the presence of adequate concentrations of specific nutritional moieties.

Autogenous mosquitoes do not require a blood-meal to complete maturation of at least the first clutch of eggs. Lea (1970) reported that a hormone from MNC of autogenous Aedes taeniohynchus was in

the hemolymph 3 days after emergence and stimulated egg development in the absence of a blood meal. Interestingly, O'Meara and Evans (1977) found that some, but not all, autogenous Aedes taeniorhynchus require mating before egg maturation. In these individuals, injection of a saline extract of male accessory glands has the same effect as mating.

Another role for JH in previtellogenic development, in addition to bringing follicles to resting stage, was presented by Flanagan and Hagedorn (1977). Their work involved the synthesis of vitellogenins by fat body when stimulated by 20-OH ecdysone, which will be discussed later. They reported that fat bodies taken from females that had been allatectomized within 1 hr of emergence would not synthesize vitellogenin when incubated with 20-OH ecdysone in vitro. Responsiveness of fat bodies of allatectomized females was restored by topical application of JH or reimplantation of CA. Flanagan and Hagedorn (1977) concluded that adult exposure to JH is required by fat body to become competent to synthesize vitellogenin in response to 20-OH ecdysone.

Hormonal Regulation of Vitellogenic Development

Egg Development Neurosecretory Hormone. By use of ablation and implantation experiments with MNC and the corpora cardiaca (CC), which are located along the aorta in the head, Lea (1967, 1970, 1972) demonstrated that in anautogenous mosquitoes prior to blood-feeding, the MNC produce a factor which is stored in the CC, and is released soon after a blood-meal. Removal of these organs several days after emergence, and hence after the proximate follicles have reached the resting stage, prevents egg maturation subsequent to a blood meal. Lea (1972), via parabiosis experiments, determined that this factor, called egg development

neurosecretory hormone (EDNH), was released in response to a hemolymph-borne factor after blood-feeding.

Ecdysteroids. The discovery by Spielman et al. (1971) that injection of microgram quantities of 20-OH ecdysone initiated vitellogenic development in mosquito eggs led to a continuing intensive research effort to clarify the role of this hormone. The data from this effort are not all mutually supportive and the exact role of ecdysteroids is still unclear.

Hagedorn and Fallon (1973) and Hagedorn (1974) reported that fat bodies from female mosquitoes that had been ovariectomized and then fed blood did not synthesize vitellogenins, and that ovaries taken from blood-fed females stimulated synthesis of vitellogenins by fat bodies cultured in vitro. This suggested the ovary as a source of a substance required to stimulate vitellogenin synthesis.

Fallon et al. (1974) showed that fat bodies removed from mosquitoes that had been injected with 5 μg of 20-OH ecdysone synthesized vitellogenin in culture. They also reported that fat bodies from unfed females when incubated with 10^{-4}M 20-OH ecdysone produced vitellogenin in amounts comparable to those of fat bodies excised from females 18 hr after feeding.

Further evidence that 20-OH ecdysone is the vitellogenic hormone was obtained by Hagedorn et al. (1975). This included a profile of endogenous whole-body ecdysteroid in blood-fed females. A rise in ecdysteroid levels begins at approximately 10 hr after blood feeding reaching a peak at 16 hr. This correlated with maximum ability to stimulate fat body in vitro by ovaries removed 18 hr after blood-feeding relative to other times. The material secreted by such ovaries was determined to be ecdysone via radioimmunoassay (RIA) and high pressure

liquid chromatography. The material obtained from whole body extracts was identified as 20-OH ecdysone. Hagedorn et al. (1975) hypothesized that after blood-feeding the ovary secretes ecdysone, which is hydroxylated to 20-OH ecdysone by the fat body. The fat body is then stimulated by 20-OH ecdysone to synthesize vitellogenin.

That hypothesis ignores the sequence of events following blood-feeding as reported by Hagedorn et al. (1973) and Hagedorn et al. (1975). According to Hagedorn et al (1973) fat body RNA begins to increase about 2 hr after blood-feeding and is followed by the beginning of vitellogenin synthesis 1-2 hr later. Hagedorn et al. (1975) determined that a significant increase in ecdysteroid content in whole females did not occur until 10 hr after blood-feeding, although there was a small peak at 4 hr which was not statistically significant. If this information is correct, then 20-OH ecdysone cannot be the trigger for synthesis of vitellogenins.

Borovsky and Van Handel (1977) criticized the previous work for use of massive dosages of 20-OH ecdysone for in vivo studies. Though they confirmed synthesis of vitellogenin by fat body, they also reported difficulties in the original RIA used by Hagedorn and Judson (1972). This prevented accurate determination of vitellogenin synthesis in ensuing work, bringing into question the role of 20-OH ecdysone. In a further reexamination of this hormone's function Borovsky and Van Handel (1979) tested several of the findings of the previous workers and were unable to confirm important points. Their findings were: (1) Incubation of fat body of unfed females with ovaries of blood-fed animals did not result in vitellogenin production, (2) fat body from unfed females injected with 5 μg of 20-OH ecdysone synthesized only 13% of that from

blood-fed mosquitoes -- considerably less than 80% reported by Fallon et al. (1974). (3) Fat body incubated in vitro with 10^{-3} or 10^{-5}M 20-OH ecdysone did not synthesize more vitellogenin than hormoneless controls. Separate reevaluations by Borovsky, Hagedorn and Kang, and Fuchs and Hagedorn (cited in Fuchs and Kang, 1981) also failed to confirm the ability of fat bodies from unfed mosquitoes to synthesize significant amounts of vitellogenin when incubated with 10^{-6} or 10^{-5}M 20-OH ecdysone.

Borovsky (1981) also questioned the idea that the ovaries are the source of a humoral factor which stimulates vitellogenesis. The report that ovariectomized mosquitoes were unable to synthesize vitellogenin (Hagedorn and Fallon, 1973) was confirmed by Borovsky and Van Handel (1979). However, Borovsky (1981) attributed this inability to a feedback mechanism whereby accumulation of vitellogenin in the hemolymph of insects whose eggs had matured or that had been ovariectomized caused termination of vitellogenesis. This remains an open question.

Criticism of use of large dosages of 20-OH ecdysone was lent some support when Beckemeyer and Lea (1980) published a report of a response to low dosage. They applied 20-OH ecdysone in two injections intended to mimic the after blood-meal concentrations as determined by Hagedorn et al. (1975). This resulted in separation of the penproximate follicles from the proximate follicles, but not in yolk disposition. It should be pointed out, however, that there seems no reason to believe that two pulses of hormone in physiological amounts would exactly mimic sustained release.

Using autogenous Aedes atropalpus, Kelly and Fuchs (1980) were able to give vitellogenic response to nanogram quantities of 20-OH ecdysone. Injection of 4.8 ng of the steroid into 12 hr old females that had been decapitated soon after emergence restored vitellogenesis in 100% of the insects. Vitellogenesis was not restored in isolated abdomens with 4.8 ng of hormone, but was restored to 42% of control with 5 μg. Production of mature oocytes in isolated abdomens was accomplished with 4.8 ng of 20-OH ecdysone after pretreatment with 0.5 ng JH I or by treatment with a large excess of JH I alone (Kelly et al. 1981).

From this body of work it is clear that 20-OH ecdysone is a critical component of regulation of egg maturation in mosquitoes. Though complete delineation of its functions is incomplete, there is presently evidence for roles for 20-OH ecdysone other than induction of vitellogenin synthesis. As already mentioned Beckemeyer and Lea (1980) found that small amounts caused follicle separation. Briegel and Lea (1979) indicated a function for the hormone in induction of midgut trypsin synthesis, and Cole and Gillet (1979) reported that it enhances blood meal retention. A particularly interesting role for 20-OH ecdysone was put forth by Schlaeger et al. (1974). This group found that dopa decarboxylase activity was increased in female mosquitoes by injection with the hormone, but not in injected males. In Drosophila it has been shown that 20-OH ecdysone induces de novo synthesis of dopa decarboxylase (Kraminsky et al., 1980). It seems likely that it fills a similar role in female mosquitoes by contributing to sclerotins in the egg chorion.

Exploitation of Host Hormone by Parasites

There are a multitude of cases wherein parasite development is synchronized with physiological changes in the host. For many of these there is evidence that suggests a response to host hormones. Due to the difficulty of demonstrating a direct hormonal stimulus, nearly all of this evidence is equivocal. The clearest demonstrations of such relationships have been for vertebrate parasites. El Mofty and Smyth (1960) suggested that the ciliate Opalina ranarum cued on the hormones of its host Rana temporaria. Rothschild and Ford (1964) showed a definite regulation of ovarian development in the European rabbit flea Spillopsyllus cuniculi by the steroid hormones of its host. This study dealt with ectoparasites, which are amenable to experimentation. The evidence for such relationships among symbiotes of invertebrate hosts is less compelling. It is with these that this review will deal.

Fungi

Thus far, only one study has been published dealing with responses of fungal symbiotes to host hormones. Sutton et al. (1979) treated three insect-associated species of fungi with 20-OH ecdysone, JH III, farnesol, and cholesterol in vitro. Of these species, Beauveria bassiana showed enhanced mycelial growth with JH III and, to a lesser extent, with 20-hydroxyecdysone, Nomuraea rileyi showed growth enhancement with 20-hydroxyecdysone, and Tritirachium dependens responded to none of the compounds. The biological significance of these findings is unclear. There are no in vivo data correlating hormone levels with fungal growth rates; nor is there any evidence that effects observed were other than nutritional.

Protozoa

The biological literature holds several reports of purported responses to host hormones by parasitic Protozoa. In none of these cases was the response proven to be a direct one. Establishment of direct causation is a very exacting task, while the elimination of hormonal implication can be quite simple. A case of the latter was the decapitation of Aedes aegypti immediately after a blood meal by Rozeboom (1961). This procedure eliminated gonadotropic hormones, but did not interfere with the development of malarial parasites to the oocyst stage. In cases where interference with host hormones also interferes with parasitic development, the alternative approach of hormone application is employed. This too gives equivocal results.

Direct treatment was used by Ilan et al. (1969) to assess the effects of JH in vitro on Crithidia fasciulata, a trypanosomatid that inhabits mosquito hindguts. They reported that JH had a reversible growth retardant effect on the flagellate. Riddiford (1975) criticized this work for the use of a JH preparation containing chlorinated compounds. This raised the question of toxic response vs. bona fide JH response. In either case it is difficult to see any biological relevance in this study. When not bound to its specific carrier protein, JH is rapidly hydrolyzed to an inactive acid-diol and conjugated with sulfates for excretion (Riddiford and Truman, 1978). It is therefore unlikely that C. fasiculata would encounter significant quantities of JH in nature.

The close synchrony of development between the eugregarine Schneideria schneiderae and its host Trichosia pubescens, a sciarid,

has been attributed to parasite use of host hormonal cues (Malavasi et al., 1976). The gregarine trophozoites in fly larvae are released from the gastric caeca cells and migrate to the gut lumen where they undergo syzygy followed by gamontocyst formation. Within the gamontocyst, gametogenesis, fertilization, and formation of the sporozoites takes place. This series of events occurs between formation of the host prepupa and the completion of the pupation, thereby assuring the presence of sporozoites for release at the time of Trichosia eclosion. The authors used injections of 20-OH ecdysone to accelerate pupation of the fly and development of Schneideria. They also used ligatures to isolate neurosecretory centers and prevent pupation. This also restricted release of gregarines from caecal cells. From this it was concluded that symbiotes respond to ecdysterone by leaving the interior of the cells of the intestinal caeca, but that the response is not necessarily direct. Because these experiments simply accelerated or prevented the pupation process with corresponding parasite behavior, the role of 20-OH ecdysone remains unclear.

A much clearer hormonal implication in parasite development cuing was achieved by Durchon and Vivier (1964). In this case the eugregarine Diplauxis hatti forms mature cysts when its host, the polychaete Perinereis cultrifera, completes epitoky (formation of a reproductive individual differing in secondary sexual characteristics from the non-sexual form). Decerebrate polychaete sections undergo epitoky with associated formation of parasite cysts whereas implantation of brains into such sections suppresses both epitoky and cyst formation. In their elegant critical experiment Durchon and Vivier severed the posterior regions of worms in the early stages of epitokic transition which had

gregarines in syzygy. These were then grafted onto intact worms in the non-epitokous condition. In the grafted regions epitoky proceeded despite the presence of brain secretions, but parasite development did not proceed beyond syzygy. It appears clear that a humoral factor secreted by the brain is required for suppression of mature cyst formation in D. hatti and that the morphological and physiological conditions for epitoky are not involved.

The classic work in this vein was conducted primarily by L. R. Cleveland who, for 35 years, studied the gut flagellates of the wood-eating cockroach, Cryptocercus punctulatus. These mutalistic symbiotes represent 9 families, 14 genera, and over 30 species (Cleveland, 1959). All enter sexual reproductive cycles during the host's molting period, an annual event in later instars, but the various protozoan species respond at different stages of the cycle (Cleveland, 1947). Cleveland and Burke (1960) transferred the flagellates to defaunate hosts and found that the sexual cycles were accelerated when the new host was closer to the molting period than the donor host and decelerated when the recipient host was farther from its molt than the donor. The culminating paper in this study deals with the effect of ecdysone on the sexual processes (Cleveland and Burke, 1960). Injection of ecdysone into the host induces acceleration of gametogenesis in all the flagellate species and retards or prevents zygotic meiosis in three genera only. These hormonal effects are duplicated in adult hosts, which do not respond to the hormone by entering molts, as nymphs do. The authors squeezed the abdomens of the cockroaches to remove ecdysone and found that this both inhibited ecdysis in the host and gametogenesis in the flagellates

as well as allowing zygotic meiosis to proceed. It was concluded that gametogenesis and inhibition of meiosis in the flagellates is a response to ecdysone and that variation among species in the type of entry into gametogenesis is due to variation in sensitivity to the hormone. It was further suggested that the response is a direct one since it was found in adult hosts that were injected with ecdysone and did not enter a supernumerary molt, and since the response by some flagellate species is more rapid than the detectible response by their host.

Cleveland's study was elegant in many ways but has some rather serious deficiencies. The ecdysone removal experiments would be more convincing if, rather than squeezing the cockroach abdomens, the authors had removed the prothoracic glands. More importantly, ecdysone was injected into the host's abdominal cavity or thorax when the flagellates are resident in the hindgut. It would be interesting to see if enemas, which would bring them into direct contact with the hormone, would have the same effect. Furthermore, it would have been of interest to attempt treating the flagellates in vitro. The techniques used leave open the possibility of a secondary response to a substance produced by the insect after ecdysone secretion, or a response to excreted ecdysteroid breakdown products.

Nematodes

In their review of endocrine relationships between nematodes and insect hosts, Davey and Hominick (1973) conclude that although some suggestive observations have been made, there is no rigorous evidence for hormonal involvement in the synchrony often seen between the development of the nematodes and that of their hosts. Several examples

of synchrony of host and parasite development are found among nematodes infecting diapausing insects. Cessation of development during host diapause has been demonstrated for Sphaerularia bombi in Bombus spp. (Stein, 1956), for Howardula sp. in Phyllotreta (Jourdheuil, 1960, as cited by Davey and Hominick, 1973), and for Heterotylenchus autumnalis in Musca autumnalis (Stoffolano, 1967). The involvement of hormones in these cases has been suggested, but no experiments have been done to confirm or refute this.

Where experiments have been done, the evidence for hormonal involvement is largely negative. Much data have been generated by in vitro application of insect hormones to nonentomophilic nematodes. The resultant responses are apparently nonspecific and lack meaning in the context of host-parasite interactions. Where entomophilic nematodes have been studied the results have been either negative or contradicted in later work. Nadakal and Nayar (1968) and Gordon (1970) reported reduced fecundity in thelostomatides in the hindguts of cockroaches that had been subjected to surgical manipulation of their endocrine organs. Hominick and Davey (1972) showed that those effects were explicable on the basis of a reduced level of nutrition for the operated cockroaches. Yoeli et al. (1962) and Gwadz and Spielman (1974) employed surgical disruption of the cycle of gonadotropic hormones of mosquitoes to demonstrate that these hormones do not affect the development of Dirofilaria immitis or Brugia pahangi. It appears that the most fertile area of research on response to host hormones by parasitic nematodes would be among those infecting diapausing insects.

Parasitoid Insects

There are thousands of insect species which are endoparasitic on other insects. Synchrony of development between parasitoid and host has been documented for a substantial number of these species (Fisher, 1971). Given the commonality of developmental hormones among insects this system is a logical one to scrutinize for exploitation of host hormones by symbiotes.

While host hormones have been implicated in the development of several parasitoids it is clear that their effects are not always direct ones. Corbet (1968) demonstrated that resumption of development of the ichneumonid Nemeritis canescens in the final instar of its host Ephestia kuehniella is a response to changes in the nutrient quality of host hemolymph and therefore only indirectly to hormones.

Experimental implication of host hormones in parasitoid development began with Schneider (1951). He investigated Diplazon fissorius, an ichneumonid that parasitizes syrphid flies and does not develop beyond the first instar until the host has pupated. By transplanting first instar wasps into hosts at different stages of development than the donor, or into host species with different development cycles, Schneider was able to advance or retard development of the parasitoids. He suggested that activation of Diplazon larvae was directed by the hormones associated with host pupation.

Schoonhoven (1962) conducted a detailed study on the synchronous development of Eucarcelia rutilla and its geometrid host Bupalus pimarius. In this case, chilling promotes termination of diapause in both species, as does parabiosis with a chilled moth pupa. Furthermore the fly larvae will not develop if the host abdomen is isolated

immediately after removal from the cold. Isolation of abdomens 5 days after removal does not prevent parasitoid development. This indicates that the prothoracic glands of the host are no longer needed, and that they have secreted enough ecdysteroid to stimulate development of the fly. Schoonhoven (1962) believes that the parasitoid is directly activated by the host during the period of endocrine activity preceding the host's adult development, and that ecdysone is the likely activating substance.

There are two contradictory findings in Schoonhoven's data. First, in 5 of 25 parabiosis replicates adult moths emerged with no development of their parasitoids. Second, in an experiment using decerebration of the host to create "permanent pupae" 23 of 68 parasitoids completed development in spite of lack of host prothoracic gland activity. These data cloud the issue of the host's role. In vitro experiments would have been useful to clarify the matter.

In vitro studies, while extremely useful, are not necessarily definitive in hormonal studies. Nenon (1972a, b) cultured the polyembryonic wasp Ageniapis fusicollis to determine the effects of exogenous hormones on this parasitoid. He found that survival of both embryo and larvae was prolonged by addition of JH and ecdysone, but development was not significantly different in hormonal treatments from that of survivors of hormoneless treatments.

A rather unusual approach to the question of parasitoid-host developmental synchrony was taken by Cals-Usciati (1969, 1975). She used γ-radiation as well as ligations to suspend the development of the Mediterranean fruit fly Ceratitis capitata and its braconid parasitoid Opius concolor. Ecdysone was then injected to stimulate host pupation which

was followed by Opius's first larval molt. Here again the question of direct vs. secondary response is unanswered.

Recent work by Lawrence (1982) demonstrated a dependence of the braconid Biosteres longicaudatus on the milieu of pupating host, Anastrepha suspensa, to initiate a molt to the second instar. Parasitoid larval molts were prevented by ligaturing the host posterior to the ring gland prior to the critical period for hormones in control of pupation. Further evidence of the need for hemolymph-borne factor was provided by parabiosis of Anastrepha pupae to parasitized larvae. This procedure induced pupation of the host larvae and molting of the parasitoids.

Egg hatch in B. longicaudatus appears to be also under host humoral control (Lawrence, 1982). Eggs implanted into postcritical period last instar hosts had delayed hatch relative to those implanted in precritical hosts. This is more likely to be due to osmolality changes than ecdysteroids, which can't penetrate the chorion. Resolution of host hormone effects on B. longicaudatus should improve with the results from in vitro studies.

Baronio and Sehnal (1980) claim to have proven a direct effect of host hormones on the development of the tachinid parasitoid Gonia cinerascens. Under natural conditions the parasitoid molts to the second instar and migrates to the exuvial space when the host prepares for pupation, but never at host larval molts. In this case, as in the others discussed, manipulations performed to alter the developmental cycle of the host also altered the development of the parasitoid, thereby maintaining synchrony. For example, application of JH II or a JH analogue to last instar hosts resulted in larval-pupal intermediates

at molting, with fly larvae molting to second instars only if pupal characteristics dominated. The author's claim of direct response rests on implantation of first instar G. cinerascens into pupae of Galleria mellonella. Such implants molt to the second instar and migrate to the exuvial space at the time of adult molt. The authors see this as proof of a direct response to ecdysteroids, which is suppressed by JH in larval molts. Surely the other physiological changes associated with pupal and adult moths must be considered.

Certainly, there is abundant evidence for a role of host hormones in regulation of symbiote development. Clear proof of a direct role for these hormones is generally very difficult to obtain. Indeed, no such proof exists for internal symbiotes. When this proof comes, it will doubtless be the result of rigorous in vitro follow-up to in vivo experiments.

The Biology of Dimorphic Microsporidia

The existence of dimorphic microsporidia was suspected by Maddox (1966) during his work with Vairimorpha necatrix. It was first documented by Hazard and Weiser (1968), who studied Parathelohania spp. in mosquito hosts. Up to that point the known morphs of such microsporidia were described as individual species. Soon thereafter it became clear that dimorphism was a widespread phenomenon among the Microspora. All the dimorphic species known to date are included in the order Microsporidia according to the classification of Sprague (1977).

The species studied by Hazard and Weiser (1968) are illustrative of the biology of most known dimorphic microsporidia, especially those

of the family Theohaniidae as described by Hazard and Oldacre (1975). In these two cases binucleate spores similar to the spores of the genus Nosema are formed in adult female mosquito hosts and function in transovarial transmission. The second spore type is uninucleate and believed to be haploid (Hazard et al., 1979). These spores are formed in groups of eight within pansporoblast membranes from octonucleate sporonts and are therefore often referred to as octospores. Uninucleate spores are formed in larvae, which usually succumb to the infection. The destiny of these spores in nature is a mystery. Attempts to infect healthy hosts with spores of this type have been generally unsuccessful (Kellen et al., 1965; Chapman, 1974). Reports of per os transmission with uninucleate spores (Bailey et al., 1967; Kellen and Lipa, 1960) are unconfirmed and suspected to be erroneous (Hazard, personal communication.

The microsporidia that are known to be dimorphic fall into the families Thelohaniidae (sensu Hazard and Oldacre, 1975) and Burenellidae as well as the genus Hazardia whose familial affinity is controversial (Sprague, 1977; Weiser, 1977) and two species whose taxonomic status is unsettled. None are known to occur in hosts outside of the Arthropoda. It is certain that many more species will be found to be dimorphic when knowledge of their life cycles is improved. This is especially true among those species whose described spores are uninucleate and nontransmissible by known procedures and species described by Thelohania that infect Lepidoptera.

Transmission

Two primary routes of transmission are known for dimorphic microsporidia. Transovarial transmission is the only means that has been documented for many species. In a few other species only per os transmission is known. The only published accounts of both types of transmission occurring in a single species probably result from exposing congeniality infected mosquito larvae to spores (Hazard, personal communication).

It was Pasteur (1870, as cited in Fine, 1975) who made the first observations on vertical (hereditary) transmission of microsporidia, the nature of which he was unaware. The earliest report of vertical transmission of a known microsporidium was that of Marchoux and Simond (1906) who studied the monomorphic species Nosema stegomiae in the mosquito Aedes aegypti. Canning and Hulls (1970) studed the closely related species N. algerae in Anopheles stephensi and found that vertical transmission occurred normally but was prevented by surface sterilizing eggs. Transmission appears to have taken place by the transovum route (via spores on egg surfaces). Zimmack et al. (1954) and Zimmack and Brindley (1957) were first to document true transovarial (inside the egg) transmission of microsporidia. They examined ovarian tissue and employed surface sterilization to demonstrate that N. pyraustae parasites were, in fact, transmitted within the egg of the European corn borer Ostrinia nubilalis.

Vertical transmission occurs in the dimorphic genera Amblyospora and Parathelohania, as well as in a number of other genera which are almost certainly dimorphic, but are lacking descriptions of the meronts and spores that are involved in egg infection. Kellen and Wills (1962)

reported transovarial transmission in Amblyospora californica, a parasite of Culex tarsalis. They did not examine parental females however, and were unaware of the dimorphic nature of the organism. Evidence was later found for vertical transmission in many species of Thelohaniidae (Kellen et al., 1965, 1966; Chapman et al., 1966).

Dimorphism and its significance for transovarial transmission among thelohaniids was described in part by Hazard and Weiser (1968) in a study of Parathelohania obesa and P. anophelis in Anopheles spp. They found that in adult female mosquitoes, plasmodia of both species in oenocytes respond to a host blood meal by giving rise to binucleate spores that lack a pansporoblast membrane. This occurs in the hemocoel with P. obesa and in the ovary with P. anophelis. It was not determined whether the binucleate spores or the plasmodia were responsible for infecting the developing eggs. This point was clarified by Andreadis and Hall (1979a) who found the binucleate spores of the undescribed Amblyospora species in Culex salinarius were short-lived and extruded their sporoplasms which were believed to enter the eggs. The mechanism of entry has yet to be determined. In both Amblyospora and Parathelohania some of the parasites remain dormant after a given host blood meal to allow for infection of eggs developed in later gonadotropic cycles (Hazard and Weiser, 1968; Andreadis and Hall, 1979a, b).

Transmission is via the oral route in the dimorphic genera Vairimorpha, Burenella, and Hazardia, and in at least two species which will probably be assigned to Vairimorpha when their placement is settled (Maddox and Sprenkel, 1978). None of these are species specific, but Vairimorpha has been transmitted only to Lepidoptera (Maddox et al.,

1981), Burenella only to fire ants (Jouvenaz and Hazard, 1978) and Hazardia only to mosquitoes (Hazard and Fukuda, 1974). It would be of interest to determine if they are capable of infecting hosts outside of these groups.

In these three genera the sporogonic sequences of both spore types may occur within an individual host (Hazard and Fukuda, 1974; Jouvenaz and Hazard, 1978; Pilley, 1976). However, Vairimorpha and its allies form only uninucleate spores if reared at high temperature (Pilley, 1976; Maddox and Sprenkel, 1978). This phenomenon and mechanical means were used by Fowler and Reeves (1974) and by Maddox and Sprenkel (1978) to separate spore types for infection studies. Both groups reported transmission with both uninucleate and binucleate spores. Similar studies have not been carried out for Hazardia and Burenella.

The work of Kellen and Lindegren (1971) presents other modes of transmission for Nosema plodiae. This has been found to be a dimorphic species and will probably be redescribed as Vairimorpha (Maddox and Sprenkel, 1978). Kellen and Lindegren (1971) reported transovarial transmission of N. plodiae in its host, Plodia interpunctella, but did not use histology or surface sterilization of eggs to confirm that the infection was transmitted within the egg. Transovum transmission was at least likely. These workers also found that the microsporidian was passed venerally from males to females.

Host-parasite Relationship

The host-parasite relationships for the dimorphic microsporidia that are transmissible per os are much like that for the majority of monomorphic species. There is apparently no differential development

between host sexes. The tissue invaded is primarily fat body in Hazardia (Hazard and Fukuda, 1974) and in Vairimorpha (Maddox et al., 1981). Burenella is somewhat different. Here uninucleate spores are formed in fat body, but binucleate spores form in epidermal tissue (Jouvenaz and Hazard, 1978). This disease is lethal at the pupal stage and has not been observed in adults. Hazardia kills many infected mosquitoes as larvae, but those with light infections survive to become adults (Hazard and Fukuda, 1974). Lethality of Vairimorpha necatrix is dose dependent and decreases with increased age of the host at the time of infection. Death is often due to septicemia resulting from damage to the gut wall caused by the polar filaments of germinating microsporidian spores (Maddox et al., 1981).

The Thelohaniidae of mosquitoes, which are probably all dimorphic, have been characterized according to their relationship to their hosts by Kellen et al. (1965, 1966) and Chapman et al. (1966). The categories are as follows:

Type I. Sporogony takes place in males, but not in females. Males die as larvae, but females survive to transmit parasites transovarially.

Type II. Sporogony takes place in both sexes, but most individuals with progressive infections are males.

Type III. Sporogony occurs in both sexes. Infections are progressive and usually fatal during the fourth larval stadium of both sexes.

Type IV. Sporogony occurs in both sexes, but areas of invasion are usually limited and do not interfere with larval development and adult survival.

These characterizations predate the work of Hazard and Weiser (1968) and hence refer only to the sporogony of uninucleate spores. The types, as originally described by Kellen et al. (1965), included tissue specificity. In light of new information the specificites given are no longer valid. For example, though only adipose tissue and oenocytes were mentioned, D. W. Hall (personal communication) has found that a thelohaniid species in Culex peccator is neurotropic.

These relationships have profound implications for the survival of the microsporidia in nature. It has been suggested that Type I relationship eliminates dependence on infective spores (Kellen et al., 1965). Andreadis and Hall (1979b) applied the mathematical model of Fine (1975) to the Type I parasitization of Culex salinarius by Amblyospora. These authors concluded that the microsporidium would rapidly disappear from its host population in the absence of a means of transmission other than transovarial. The need for an alternate route of transmission is particularly clear for Type III relationships. Lord et al. (1981) found that progeny of female Aedes taeniorhynchus, which are infected with Amblyospora polykarya, develop only uninucleate spores or are uninfected. Transovarial transmission is therefore only a one generation event, and a means of horizontal transmission must exist.

The obvious candidate for the agent of horizontal transmission is the uninucleate spore. As mentioned earlier, this spore types does not appear to be infective directly. The possible requirement for an intermediate host deserves attention. A clue to the role of these spores may be found in the recently discovered occurrence of meiosis (Hazard et al., 1979).

Sexuality

Evidence for meiosis in microsporidia was first reported when Loubè et al. (1976) published electron micrographs of synaptonemal complexes in uninucleate sporonts of Gurleya chironomi. More recently Hazard et al. (1979) gave an account of meiosis in Amblyospora and Parathelohania which included studies of chromosome squashes in addition to electron micrographs of synaptonemal complexes. The reduction division is the first nuclear division of the sporogony of uninucleate spores. These spores, then, are apparently haploid.

The uninucleate spores of Amblyospora and Parathelohania are produced in great numbers in mosquito larva, but don't infect new hosts by a known route. Since there is no evidence for karyogamy in mosquito hosts, perhaps an intermediate host is required for restoration if diploidy and acquisition of infectivity.

Meiosis has not been reported in Vairimorpha. It would be of interest to determine if the uninucleate spores of this genus are haploid. Since they are transmissible karyogamy would probably take place in the same host as meiosis.

Importance

The microsporidia play an important role in both natural and artificial regulation of host populations (Henry, 1981; McLaughlin, 1971). Among Protozoa being developed for control of pest insects, the dimorphic species Vairimorpha necatrix appears to have great promise against lepidopterous species (Maddox et al., 1981). Nosema pyraustae has shown potential for use in control of Ostrinia nubilalis (Lublinkhof et al., 1979). The thelohaniids play a role in the natural regulation

of disease vectoring mosquitoes, but evaluation of their potential for manipulation for artificial control awaits resolution of the uninucleate spore enigma (Chapman, 1974).

In a more academic vein, the dimorphic microsporida may eventually be used to shed light on the evolution of sexuality in Protozoa. If the Burenellidae are found to have meiosis and if thelohaniid uninucleate spores are transmitted, perhaps they will yield information on the requirements for sexual reproduction in single celled organisms.

The phylogeny of Microspora is far from settled and subject to some fundamental differences of interpretation (Sprague, 1977; Weiser, 1977). More and more microsporidia that were described from only one form or that were described as two separate species are being redescribed as dimorphic (Hazard and Weiser, 1968; Hazard and Oldacre, 1975; Pilley, 1976). There is no question that documentation of dimorphic life cycles is of profound importance to microsporidian taxonomy (Vavra et al., 1981).

EXPERIMENTAL DETERMINATION OF THE INDUCER OF SPORULATION OF *Amblyospora*

Introduction

The undescribed species of *Amblyospora* that infects *Culex salinarius* is a dimorphic parasite whose developmental pathway is sex dependent in accordance with the Type I host-parasite relationship of Kellen et al. (1965). In male mosquitoes diplokaryotic cells spread from oenocytes to adipose tissue where merogony is succeeded by a sporogonic sequence that results in formation of groups of 8 haploid spores within pansporoblastic membranes. The massive buildup of spores usually results in the death of infected males as fourth instar larvae. In female hosts, infections are limited to oenocytes until after sporulation, and multiplication is limited. Sporulation occurs within hypertrophied oenocyte after adult female mosquitoes feed on blood. The resultant binucleate spores extrude their sporoplasms, which effect invasion of the developing host oocytes and hence the next mosquito generation (Andreadis and Hall, 1979a).

The requirement for host blood-feeding just prior to sporulation in female mosquitoes suggests that the parasite may respond to the nutrients that are released into the hemocoel or that there may be a more intimate host-parasite relationship in which the microsporidium responds to the physiological changes associated with the host's gonadotropic cycle. In addition to blood meal nutrients, the substances known to appear in the hemolymph during this cycle are vitellogenins

and hormones, particularly egg development neurosecretory hormone (DNH) (Lea, 1972) and ecdysteroids secreted by ovaries (Hagedorn et al., 1975). The primary purpose of this study was to determine which, if any, of these factors is used by Amblyospora sp. as a cue to initiate sporulation.

Materials and Methods

Experimental Animals

Healthy and Amblyospora infected C. salinarius were colonized by James Haeger, Florida Medical Entomology Laboratory, Vero Beach, FL. Larvae were reared in 33 x 21 x 15 cm enamel pans, each with 75 individuals. One hundred milliliters of tap water and approximately 30 mg of Koi-goldfish food (Aquatrol, Inc., Anahiem, CA) was added at hatch and at 2 and 5 days posthatch. On alternate days thereafter until pupation or death of larvae, 15 ml of 10 mg/ml brewer's yeast suspension was added. Both larvae and adults were maintained at 25 ± 1°C under natural photoperiod. Caged adults were given 5% sucrose and raisins as carbohydrate sources, and females were blood-fed on guinea pigs. Since almost all males from the infected colony died during the fourth larval stadium, males from the healthy colony were used to inseminate infected females. Females were used for experiments 5 and 6 days post emergence.

Treatments

For all treatments mosquitoes were lightly anesthetized with nitrogen gas. Injections and topical applications were done with a finely drawn capillary tube calibrated to approximately 1 µl. Carbidopa and methyldopa were dissolved in saline. Cholesterol and 20-OH ecdysone were

dissolved in insect saline with 10% ethanol. All chemicals used in the treatments were purchased from Sigma Chemical Co., St. Louis, MO, except for methoprene which was obtained from Zoecon Co., Palo Alto, CA, and carbidopa which was kindly donated by Merck, Sharp and Dohme, Rahway, NJ. Methoprene and JH I were dissolved in acetone. Egg macerate was prepared by removal of ovaries from gravid mosquitoes, maceration in a ground glass tissue grinder with 0.15 M NaCl buffered to pH 6.9, and centrifugation at ±800 g to remove particulate material. Injections were made through the thoracic pleuron except when the females had been ovariectomized, when material was injected through the partially closed wounds.

Surgery

To remove the MNC and CC, the sources of EDNH, mosquitoes were decapitated, and the wound sealed with paraffin (Fig. 1). Decapitations of blood engorged individuals were done with 30 min of feeding.

Ovariectomies were performed on mosquitoes starved for 24 hr prior to surgery. After placing them in a mold of modeling clay with a thin strip of clay across the thoraxes as a restrainer, the abdomens were immersed in saline. Sharpened jeweler's forceps were then used to remove the ovaries through single ventral incisions between the 6th and 7th abdominal sternites (Fig. 2). Wounds were sealed with paraffin, and operated individuals were offered a guinea pig blood source after approximately 6 hr.

Implantations were conducted in a similar manner. Activated C. salinarius brains were taken from healthy females 1-3 hr after blood feeding. Mosquito ovaries were taken from individuals that had taken

FIGURE 1. BLOOD-FED AND DECAPITATED C. salinarius

FIGURE 2. ABDOMEN OF C. salinarius with an OVARY BEING REMOVED.

blood 9-12 hr prior to dissection. CC of Periplaneta americana were taken from late instar nymphs.

Postoperative mosquitoes were kept in cages fashioned from histological staining dishes. These were supplied with screening placed under the covers, moist paper towel lining to maintain high humidity, and raisins as a food source (Fig. 3) for mosquitoes.

Scoring

Unless otherwise indicated all individuals were scored 48 hr after treatment. Blood-feeding of infected mosquitoes results in near completion of sporulation after that length of time. Mature spores are then detectible for another 12-24 hr (Andreadis and Hall, 1979a).

Slides were prepared for screening for sporulation by smearing whole mosquitoes and staining with Giemsa stain after methanol fixation. Slides with a single spore were scored as positive. Those with at least 30 vegetative Amblyospora and no spores were scored as negative for sporulation. Spores and vegetative stages were easily distinguishable in Giemsa preparations (Fig. 4).

Microscopy Studies

For preparation of paraffin sections, mosquitoes were fixed in Zenker's fixative for 8 hr, washed overnight in running water and soaked in iodine-saturated 70% ethanol for 2-4 days. They were then taken through an ethanol series and into tertiary butanol from which they were infiltrated with paraffin (Lillie, 1965). Sectioning was carried out on an American Optical rotary microtome model 820. Sections were deparaffinized and hydrated through a xylene-ethanol series, stained

FIGURE 3. CAGES FOR POSTOPERATIVE C. salinarius

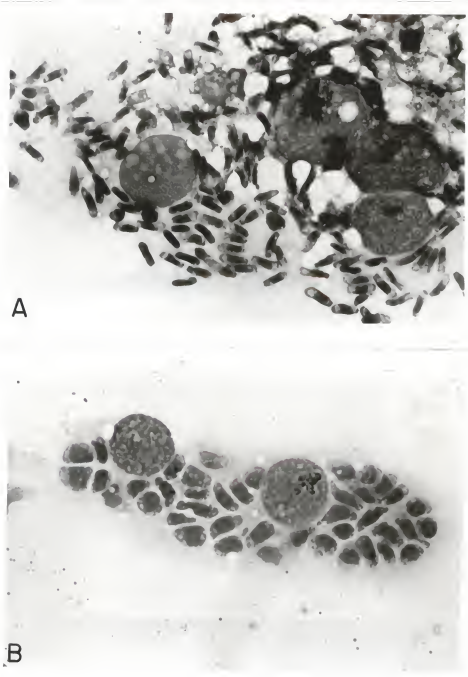

FIGURE 4. PHOTOMICROGRAPHS OF Amblyospora SP. STAINED WITH GEIMSA STAIN. A. SPORES. B. VEGETATIVE STAGES. 600X.

with the basic fuchsin nucleic acid stain technique of Horobin and Kevill-Davies (1971), and counterstained with fast green FCF (Humason, 1979). Stained sections were mounted in Flo-texx (Lerner Laboratories, Stamford, CT).

For presentation of plastic sections, specimens were dissected in 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate, pH 7.5, and fixed for 2 hr at room temperature in the dark in 2.5% glutaraldehyde, 0.1% peroxide in 0.1 M cacodylate buffer, pH 7.5 (Peracchia and Mittler, 1972). After several buffer washes, specimens were postfixed in 1% OsO_4, dehydrated in an ethanol series, en bloc stained with 0.5% uranyl acetate in 70% ethanol, and embedded in a Spurr-Epon mixture (Ellis and Avery, 1978). Sections were cut on a Sorvall MT-1. Ultrathin sections were poststained with 5% methanolic uranyl acetate, followed by lead citrate (Reynolds, 1963), and examined with an Hitachi Series 600 electron microscope at an acceleration voltage of 75 kV. Thick sections, which were not poststained, were examined and photographed by phase contrast microscopy.

Autoradiography

Autoradiographs were prepared as described by Bogoroch (1972). Infected female mosquitoes were injected 22 hr after blood-feeding with 1 µg of 1 mCi/ml, 3,4-dihydroxy (ring-2,5,6-^{3}H)phenylethylamine hydrochloride (hereinafter ^{3}H-dopamine) obtained from Amersham Corp., Arlington Hts., IL. Twenty-six hours later they were fixed and embedded in Spurr-Epon as described. Sections of 0.5-1.5 µm were placed on unsubbed slides and dipped in Kodak NTB2 diluted 1:1 with glycerol.

These were stored at 2°C for 8, 14 or 30 days prior to D-19 development and mounting in Flo-texx.

Cytochemistry

Attempts to localize phenol oxidases were carried out as described by Lewis and Knight (1977). Attempts to demonstrate catecholamines were carried out on sections of freeze-dried, paraffin-embedded mosquitoes using the formaldehyde-induced fluorescence technique of Falck (1962).

Cell Culture

To test the effect of 20-OH ecdysone on vegetative Amblyospora in vitro, oenocytes were dissected from females that had emerged in a laminar flow hood and were fed only sterilized 5% sucrose. The surfaces of the mosquitoes were disinfected by exposure to ultraviolet light directed through plastic food wrap and reflected by aluminum foil. The oenocytes were maintained for 36-48 hr in Schneider's Drosophila medium (KC Biological, Lenexa, KS) with approximately 1 μg/ml (2×10^{-6}M) 20-OH ecdysone and 100 units penicillin and 100 μg/ml streptomycin.

Results

Hormonal Manipulations

Decapitation of host mosquitoes immediately after blood feeding prevented the sporulation of Amblyospora that otherwise followed the blood meal (Table 1). This indicates that factors other than nutrients from digested blood and gut stretch stimulus trigger the spore formation process. The presence of spores of two of the decapitated controls which were not given blood may be due to autogeny which is present in a small percentage of the mosquitoes in our colony or to continuous,

TABLE 1. EFFECT OF DECAPITATION OF NEWLY BLOOD-FED *C. salinarius* ON SPORULATION OF *Amblyospora* SP.

	Vegetative stages only	Spores
Blood-fed, decapitated	24	0
Blood-fed, not decapitated	0	20
No blood, decapitated	14	2

asynchronous follicular development found in some Culex mosquitoes (Nayar and Knight, 1981).

Since vitellogenin appears in mosquito hemolymph shortly after a blood meal, this protein must be considered as a candidate for the sporulation cue for Amblyospora. The vitellin of mature mosquito eggs is immunologically indistinguishable from its vitellogenin precursor (Hagedorn et al., 1978). With this in mind and with the consideration that other egg substances are presumably among those that appear in the hemolymph during gonadotropic development, it was decided to use the soluble material from a macerate of mature eggs for injection into whole unfed hosts. A two-fold dilution of a concentration that proved lethal was used. Spores were found in only 1 of 20 treated individuals (Table 2). Again, the occurrence of autogeny may account for the presence of spores. Egg proteins appear not to induce sporulation of Amblyospora.

One approach to investigating the effects of hormones was to implant the organs responsible for their secretion. In addition to activated brains and ovaries of C. salinarius, the corpora cardiaca of nymphal Periplaneta americana were used because the latter were found by Hagedorn et al. (1979) to be even more effective than mosquito brains (the source of EDNH) in stimulating mosquito ovaries to secrete ecdysone in vitro.

None of the organ implantations induced the microsporidium to sporulate (Table 3). This may be due to damage to the organs or hosts incurred during the operations or to the inhibition of activity by the unreceptive milieu of a previtellogenic host. High mortality suggests

TABLE 2. EFFECT OF SPORULATION OF *Amblyospora* SP. OF INJECTION OF HOST EGG MACERATE INTO *C. salinarius*

	Vegetative stages only	Spores
Egg macerate	19	1
Saline	16	0

TABLE 3. EFFECT OF SPORULATION OF *Amblyospora* SP. OF ORGAN IMPLANTATION INTO *C. salinarius*

	Vegetative stages only	Spores
Activated brain	8	0
Corpora cardiaca of *P. americana*	4	0
Activated ovary	6	0
Sham-operated	5	0

the former. In either case these results may be regarded as inconclusive.

The alternate approach to the question of hormonal induction of sporulation was simply to treat infected mosquitoes with the hormones and their analogs. Since EDNH is not commercially available, indeed its exact nature is unknown, this hormone could not be used.

JH is required for the development of mosquito ovaries to the previtellogenic stage (Lea, 1963, 1969). Borovsky (1981) has speculated on a vitellogenic role for this hormone. To determine if it has an effect on Amblyospora, JH I and its more stable analog methoprene were applied topically to infected C. salinarius. JH I was ineffective at 1 µg, and methoprene was ineffective up to a dose of 6 µg which was lethal to most mosquitoes (Table 4).

Shortly after a mosquito takes a blood meal its ovaries secrete ecdysone, which is then hydroxylated by other tissues to form the more active 20-OH ecdysone. This hormone, when injected into infected mosquitoes at low doses, did not induce sporulation. At a dose of 2.5 µg all 32 of the individuals injected contained spores. At 100 ng, 20 of 23 had spores (Table 5). These are pharmacological rather than physiological doses. The fact that such a large dose is required may be explained, at least in part, by the rapid degradation of ecdysteroid in vivo (Ohtaki and Williams, 1970). It seems likely from these results that 20-OH ecdysone, or some substance whose presence is induced by it, is the cue to which Amblyospora responds by sporulation.

To see if this was a general response to steroids, a saturated solution of cholesterol was injected into infected C. salinarius. This was without effect (Table 6).

TABLE 4. EFFECT ON SPORULATION OF *Amblyospora* SP. OF TOPICAL APPLICATION OF JH I AND METHOPRENE TO *C. salinarius*

	Vegetative stages only	Spores
10 ng methoprene	20	0
60 ng methoprene	30 (5 at 72 hr)	0
1 µg methoprene	18	0
6 µg methoprene	12 (5 at 72 hr)	0
1 µg JH I	32	0
Acetone	14	0

TABLE 5. EFFECT ON SPORULATION OF *Amblyospora* SP. OF INJECTION OF 20-OH ECDYSONE INTO *C. salinarius*

	Vegetative stages only	Spores
100 pg + 250 pg at 15 hr	8	0
10 ng	14	0
100 ng	3	20
2.5 µg	0	32
Saline	28	0

TABLE 6. EFFECT ON SPORULATION OF *Amblyospora* SP. OF INJECTION ON CHOLESTEROL INTO *C. salinarius*

	Vegetative stages only	Spores
2.5 µg Cholesterol	16	0
Saline	12	0

Since the ovaries are the source of ecdysteroids in blood-fed mosquitoes it was decided to determine whether Amblyospora would sporulate in mosquitoes which were given blood after the removal of their ovaries. Sporulation was not observed in any of these ovariectomized individuals (Table 7). In one of the sham-operated controls, only vegetative stages were found. This is probably because some of the Amblyospora fail to sporulate after the first host blood meal, but do so after a subsequent blood meal thereby infecting later egg clutches. In the sham control in question, spores may have been present in the mosquito, but not smeared on the slide.

While sporulation did not occur in blood-fed females that lacked ovaries, it was possible to induce sporulation in ovariectomized mosquitoes by injection of 20-OH ecdysone (Table 7). This further supports the hypothesis that ecdysone secretion by the ovaries is required to induce spore formation in this parasite.

Twenty-OH ecdysone induces synthesis of dopa decarboxylase in insects (Kraminsky et al., 1980). This enzyme catalyzes production of catecholamines involved in sclerotization of the insect cutile and presumably the egg chorion. Furthermore the microsporidian spore wall resembles cuticle in being of protein and chitin (Varva, 1976). To see if catecholamines appearing in the hemolymph following 20-OH ecdysone are a sporulation trigger, 10 µg dopamine and 1.5 µg N-acetyl-dopamine (a saturated solution) were injected into the hosts. Neither was effective (Table 8).

To determine if the spores formed in response to 20-OH ecdysone are normal, electron micrographs were prepared from hormone-injected

TABLE 7. EFFECT ON SPORULATION OF *Amblyospora* SP. OF OVARIECTOMY OF *C. salinarius*

	Vegetative stages only	Spores
Ovariectomized and blood-fed	26	0
Sham-operated and blood-fed	1	16
Ovariectomized and injected with 2 µg 20-OH ecdysone	0	24
Ovariectomized and injected with saline	15	0

TABLE 8. EFFECT ON SPORULATION OF *Amblyospora* SP. OF INJECTION OF CATECHOLAMINES INTO *C. salinarius*

	Vegetative stages only	Spores
10 µg Dopamine	16	0
1.5 µg N-acetyldopamine	23	2
Saline	20	0

and blood-fed mosquitoes. There were no discernible differences in the ultrastructure of the resultant young spores (Fig. 5).

Attempts to stimulate sporulation by application of 20-OH ecdysone to infected oenocytes in vitro met with no success. This may be due to technique difficulties or to the inability to simulate conditions in the intact hosts at the time of sporulation.

Developmental Synchrony

If 20-OH ecdysone is a requirement for the induction of sporulation of Amblyospora, then the onset of sporulation must follow the rise in hormone level within a reasonably short time. To determine the time sequence of spore formation, infected C. salinarius females were given blood and incubated at 25 ± 0.5°C. At 4 hr intervals from 24 to 48 hr after feeding, mosquitoes were removed and prepared for microscopy.

The earliest sporoblasts detectible with light microscopy were formed 36 hr after blood-feeding. This is somewhat later than the 24 hr reported by Andreadis and Hall (1979a) who employed electron microscopy for this purpose and were therefore able to discern the earliest morphological changes associated with spore formation. In either case, the timing is such that sporulation followed the period of ovarian ecdysone secretion by an interval consistent with a causal relationship. Hagedorn et al. (1975) reported that whole body ecdysteroid levels of Aedes aegypti began to rise 10 hr after blood-feeding, peaked at 16 hr, and returned to resting level by 30 hr.

Sporulation continued through 52 hr, and spores had extruded by 72 hr. This raises the question of how the sporoplasms gain entry

FIGURE 5. ELECTRON MICROGRAPHS OF IMMATURE SPORES OF Amblyospora SP. A. 20-OH ECDYSONE INDUCED. B. BLOOD MEAL INDUCED. X 6000.

into the developing eggs. The egg chorion was readily detectible by 48 hr. Autoradiographs prepared from mosquitoes injected with tritiated dopamine showed chorion uptake of the isotope at 48 hr. (Fig. 6). This suggests that sclerotization was underway and would presumably present a formidible barrier to entry by sporoplasms. The route of entry into the developing egg remains to be resolved.

Evidence for Lack of Sclerotins in the Spore Wall

A reasonable corollary to hypothesized induction of sporulation by 20-OH ecdysone might be that the hormone is required for production of catecholamines for possible sclerotin-like proteins in the microsporidian spore wall. The spore wall resembles the insect cuticle in consisting of a protein-chitin complex (Vavra, 1976). In insects, one function of 20-OH ecdysone is induction of de novo synthesis of dopa decarboxylase (Kraminsky et al., 1980). This enzyme catalyzes the synthesis of dopamine, a compound central to sclerotization of the insect cuticle. Several approaches were taken to investigate the possibility that the microsporidian spore wall contains sclerotin-like compounds. All yielded negative data.

The first approach was an attempt to localize enzymes associated with sclerotization. Attempts to demonstrate phenol oxidases and dopa decarboxylase were unsuccessful.

Injection of dopa decarboxylase inhibitors was more evidential. Carbidopa, a potent inhibitor of dopa decarboxylase was injected at 0.7 µg per female 12 hr after blood-feeding. It was shown by Schlaeger and Fuchs (1974) and confirmed during this study that this amount prevents hardening and darkening of mosquito eggs, but it did

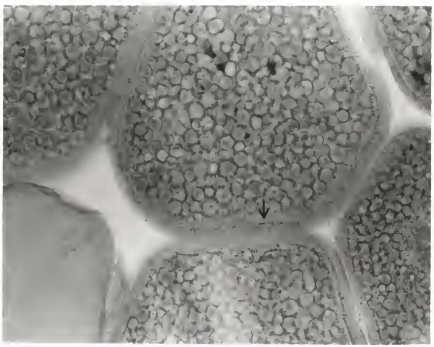

FIGURE 6. AUTORADIOGRAPH OF C. salinarius OOCYTES SHOWING SILVER GRAINS IN DEVELOPING CHORION (arrow). 500X

not prevent sporulation of Amblyospora in 21 treated insects. Likewise, injection of 2.0 µg of methyldopa was without effect in 20 replicates.

Perhaps the strongest indication of the absence of sclerotins was obtained from autoradiographs. Tritiated dopamine was injected at 22 hr after blood-feeding. Treated females were fixed with glutaraldehyde 26 hr later and embedded in plastic. This time frame allowed exposure of the parasites to ^{3}H-dopamine from just prior to the onset of sporulation until the appearance of the first mature spores. During exposure, the mosquito cuticle, including that of tracheae, took up the label, but Amblyospora did not (Fig. 7). Some of the host eggs also showed label intake in the chorion region, indicating the beginning of sequestering of dopamine preparatory to egg sclerotization (Fig. 6).

Although these results are negative, taken together they constitute a strong indication that sclerotin-like proteins are not present in Amblyospora.

Discussion

It can be concluded from the findings of this study that the addition of 20-OH ecdysone to the hemolymph of C. salinarius induces sporulation of its parasite, Amblyospora sp. From this I suggest that ecdysteroids may be required for this process. There is, however, no evidence that the effect of ecdysteroids on the microsporidium is direct or independent of the physiological changes that occur in the host as a response to 20-OH ecdysone during gonadotropic development. With the exception of EDNH, which is believed to be the inducer of

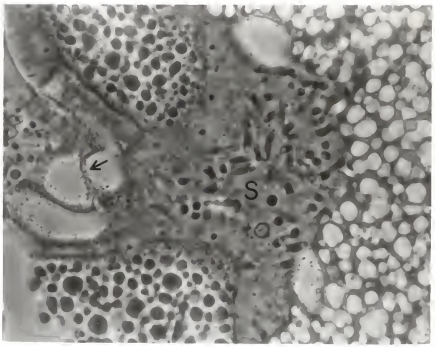

FIGURE 7. AUTORADIOGRAPH OF Amblyospora SP. SPORES AND C. salinarius TRACHEAE WITH SILVER GRAINS (arrow) S-SPORES. 750X

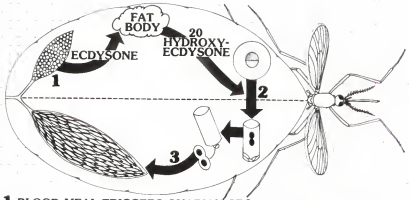
FAT BODY
20 HYDROXY-ECDYSONE
ECDYSONE
1
2
3
1 BLOOD MEAL TRIGGERS OVARIAN SECRETION OF ECDYSONE
2 SPORULATION IS COMPLETED BY 48 HR. POST BLOOD MEAL IN RESPONSE TO 20 HYDROXYECDYSONE
3 SPOROPLASM INFECTS DEVELOPING EGGS

FIGURE 8. PROPOSED SCHEME FOR INDUCTION OF SPORULATION OF Amblyospora SP. IN C. salinarius.

ecdysone secretion (Hagedorn et al., 1979), all the substances known to appear of increase in mosquitoes when undergoing egg maturation were tested for their influence on Amblyospora. Only 20-OH ecdysone showed any effect. A proposed scheme for the induction of spore formation by this hormone is presented in Figure 8.

There were a few instances in which spores were found in a small minority of individuals given a treatment other than blood-feeding or injection of 20-OH ecdysone. As mentioned, the explanation for this may be the occurrence of continuous, asynchronous follicular development reported in some Culex mosquitoes by Nayar and Knight (1981). It was not exception to find a number of mature eggs in dissected ovaries of unfed Culex salinarius. Six such individuals were smeared to screen for spores, but none were found. It could be that by the time the eggs had reached maturity, any spores present had germinated.

The large size of the dosage of 20-OH ecdysone needed to induce sporulation may, as stated, be due to its rapid breakdown in vivo. However, the 100 ng figure is considerably less than the µg quantities required to induce vitellogenesis in the host (Fuchs and Kang, 1981). Certainly the inability of the parasites to form spores in insects that had been ovariectomized and fed blood lends credence to the theory that ecdysteroids are a requirement for sporulation. This requirement may not be independent of other factors. It is possible that the size of the dose of exogenous hormone is a reflection of the lack of such a factor.

The spore wall of microsporidia is similar to the insect integument in that the outer region is primarily proteinaceous and the inner region

is primarily chitin (Vavra, 1976). Since Amblyospora sporulates when components of insect sclerotin are being synthesized by its host in response to 20-OH ecdysone (Schlaeger et al., 1974), it seems reasonable that synthesis of catecholamines by either the host or the parasite might be a requirement for spore formation. The results obtained in this study suggest otherwise. To be sure, the evidence is all negative and hence not constitutive of rigorous proof, but the suggestion seems a strong one. Maurand and Loubès (1973) reported cytochemical detection of sulfhydryl groups in the walls of sporoblasts of four species of microsporidia. In two of these the sulfhydryl groups were lost in mature spores. Perhaps the structural integrity of microsporidian spore walls is primarily due to disulfide bonds in proteins.

In evolutionary terms, the ability of a parasite to synchronize its development to that of its host through the host's hormones suggests a long and successful relationship. In the case of Amblyospora in C. salinarius this presumably allows the parasite to continue merogony up to the time when it must sporulate in order to invade developing eggs to effect transovarial transmission.

There are two documented cases of protozoan symbiotes of insects in which the host hormones have been implicated as developmental cues for the protozoans. The gut flagellates of the wood-eating cockroach Cryptocercus enter sexual cycles when the host prepares to molt (Cleveland et al., 1960). Evidence suggests that this may be due to the appearance of degradation products of 20-OH ecdysone in the hindgut milieu. In this system, the adaptive advantage lies in the coordination of symbiotes with others of their species to maximize fertilization.

In the case of the gregarine Schneideria schneiderae, coordination both with others of the species and with the sciarid host Trichosia pubescens provides adaptive benefit. Here, the parasites leave caecal cells and enter sexual cycles at the onset of host pupation. This coordination ensures both maximal fertilization and the presence of the disseminative sporozooit stage when host adults emerge. This process, including host pupation, was induced by injection of 20-OH ecdysone (Malavasi et al., 1976).

For Amblyospora sp. the synchrony with its host is even more exacting than for Schneideria. Transmission from female mosquitoes can only be achieved if spores are formed after blood-feeding. This requirement for exact synchrony does not apply to vertical transmission of monomorphic microsporidia. The spores of these species are generally long-lived and often transmitted on the outside of eggs (Canning and Hulls, 1970). In some monomorphic species, meronts infect eggs and sporulation takes place after infection (Thomson, 1958; Brooks, 1968). Perhaps the synchrony and hormonal dependence observed in Amblyospora sp. represents the evolutionary pinnacle among the Microspora.

The research presented here leaves open several avenues for continued work. The apparent lack of sclerotins in Amblyospora indicates that there may be a different mechanism to account for the structural integrity of the spore wall. Perhaps attempts to localize disulfide bonds would be useful. The sclerotization of host eggs prior to completion of sporulation raises the question of the route of entry of sporoplasms into the egg. An investigation of this matter would

probably involve an extended effort at sectioning material. Finally, it might be worthwhile to pursue further the induction of sporulation in vitro. Perhaps refinement of the technique or addition of other substances, such as hormones, would solve the problem.

LITERATURE CITED

1. Anderson, W. A. and Spielman, A. 1971. Permeability of the ovarian follicle of Aedes aegypti mosquitoes. J. Cell. Biol., 50, 201-221.

2. Andreadis, T. G. and Hall, D. W. 1979a. Development ultrastructure, and mode of transmission of Amblyospora sp. (Microspora) in the mosquito. J. Protozool., 26, 444-452.

3. Andreadis, T. G. and Hall, D. W. 1979b. Significance of transovarial infections of Amblyospora sp. (Microspora: Thelohaniidae) in relation to parasite maintenance in the mosquito Culex salinarius. J. Invertebr. Pathol., 34, 152-157.

4. Bailey, D. L., Barnes, W. M. and Dewey, R. W. 1967. Stempellia magna (Nosematidae: Microsporidia) in Culex restuans Theobald from Virginia. Mosq. News, 27, 111-114.

5. Baronio, P. and Sehnal, F. 1980. Dependence of the parasitoid Gonia cinerascens on the hormones of its lepidopterous hosts. J. Insect Physiol., 26, 619-626.

6. Beckemeyer, E. F. and Lea, A. O. 1980. Induction of follicle separation in the mosquito by physiological amounts of ecdysterone. Science, 209, 815-821.

7. Bellamy, R. E. and Bracken, G. K. 1971. Quantitative aspects of ovarian development in mosquitoes. Can. Entomol., 103, 763-773.

8. Bogoroch, R. 1972. Liquid emulsion autoradiography. In "Autoradiography for Biologists" (P. B. Gahan, ed.), Academic Press, New York, pp. 66-94.

9. Borovsky, D. 1981. Feedback regulation of vitellogenin synthesis in Aedes aegypti and Aedes atropalpus. Insect Biochem., 11, 207-213.

10. Borovsky, D. and Van Handel, E. 1977. A specific radioimmunoassay for vitellogenesis in mosquitoes. J. Insect Physiol., 23, 655-658.

11. Borovsky, D. and Van Handel, E. 1979. Does ovarian ecdysone stimulate mosquitoes to synthesize vitellogenin? J. Insect Physiol., 25, 861-865.

12. Briegel, H. and Lea, A. O. 1979. Influence of the endocrine system on tryptic activity in female Aedes aegypti. J. Insect Physiol., 25, 227-230.

13. Brooks, W. M. 1968. Transovarial transmission of Nosema heliothidis in corn earworm, Heliothis zea. J. Invertebr. Pathol., 11, 510-512.

14. Cals-Usciati, J. 1969. Influence de l'état physiologique de l'hôte Ceratitis capitata Wied. (Diptera) sur le développement du parasite Opius concolor Szepl. (Hymenoptera). C.R. Hebd. Seances Acad. Sci., 269, 342-344.

15. Cals-Usciati, J. 1975. Répercussion de la modification du cycle normal de Ceratitus capitata Wied. (Diptere, Trypetidae), pas irradiation γ et injection d'ecdysone, sur le développement de son parasite Opius concolor Szepl. (Hyménoptère, Braconidae). C.R. Hebd. Seances Acad. Sci., 281, 275-278.

16. Canning, E. U. and Hulls, R. H. 1970. A microsporidian infection of Anopheles gambiae Giles, from Tanzania, interpretation of its mode of transmission and notes on Nosema infections in mosquitoes. J. Protozool., 17, 531-539.

17. Chapman, H. C. 1974. Biological control of mosquito larvae. Annu. Rev. Entomol., 19, 33-59.

18. Chapman, H. C., Woodard, D. B., Kellen, W. R. and Clark, T. B. 1966. Host-parasite relationships of Thelohania associated with mosquitoes in Louisiana (Nosematidae: Microsporidia). J. Invertebr. Pathol., 8, 452-456.

19. Clements, A. N. 1963. "The Physiology of Mosquitoes" vol. 17, International Series of Monographs on Pure and Applied Biology. Macmillan Co., New York.

20. Cleveland, L. R. 1947. Sex produced in the Protozoa of Cryptocercus by molting. Science, 105, 16-17.

21. Cleveland, L. R. 1959. Sex induced with ecdysone. Proc. Nat. Acad. Sci. USA, 45, 747-753.

22. Cleveland, L. R. and Burke, A. W. 1960. Modifications induced in the sexual cycles of the protozoa of Cryptocercus by change of host. J. Protozool., 7, 240-245.

23. Cleveland, L. R., Burke, A. W. and Karlson, P. 1960. Ecdysone induced modifications in the sexual cycles of the protozoa of Cryptocercus. J. Protozool., 7, 229-230.

24. Cole, S. J. and Gillet, J. D. 1979. The influence of the brain hormone on retention of blood in the midgut of the mosquito Aedes aegypti (L.) III. The involvement of the ovaries and ecdysone. Proc. R. Soc. Lond. Ser. B., 205, 411-422.

25. Corbet, S. A. 1968. The influence of Ephestia kuehniella on the development of its parasite Nemeritis canescens. J. Exp. Biol., 48, 291-304.

26. Davey, K. G. and Hominick, W. M. 1973. Endocrine relationships between nematodes and their insect hosts -- A review. Exp. Parasitol., 33, 212-225.

27. Durchon, M. and Vivier, E. 1964. Influence des sécrétions endocrines sur le cycle des Gregarines chez les Néreidiens. Ann. Endocrinol., 25, 43-48.

28. Ellis, E. A. and Avery, S. W. 1978. Resin formulations incorporating Epon 812 into a low viscosity embedding medium. Proc. Southeast Electron Microscopy Soc., 1, 20.

29. El Mofty, M. and Smyth, J. D. 1960. Endocrine control of sexual reproduction in Opalina ranarum parasitic in Rana temporaria. Nature, Lond., 186, 550.

30. Falck, B. 1962. Observations on the possibilities of the cellular localization of monoamines by a fluorescence method. Acta Physiol. Scand., 56, Suppl. 197, 1-25.

31. Fallon, A. M., Hagedorn, H. H., Wyatt, G. R. and Laufer, H. 1974. Activation of vitellogenin synthesis in the mosquito Aedes aegypti by ecdysone. J. Insect. Physiol., 20, 1815-1823.

32. Feinsod, F. M. and Spielman, A. 1980a. Nutrient-mediated juvenile hormone secretion in mosquitoes. J. Insect Physiol., 26, 113-117.

33. Feinsod, F. M. and Spielman, A. 1980b. Independently regulated juvenile hormone activity and vitellogenesis in mosquitoes. J. Insect Physiol., 26, 829-832.

34. Fine, P.E.M. 1975. Vectors and vertical transmission: An epidemiologic perspective. Ann. N.Y. Acad. Sci., 266, 173-194.

35. Fisher, R. C. 1971. Aspects of the physiology of endoparasitic Hymenoptera. Biol. Rev., 46, 243-278.

36. Flanagan, T. R. and Hagedorn, H. H. 1977. Vitellogenin synthesis in the mosquito: The role of juvenile hormone in the development of responsiveness to ecdysone. Physiol. Entomol., 2, 173-178.

37. Fowler, J. L. and Reeves, E. L. 1974. Spore dimorphism in a microsporidian isolate. J. Protozool., 21, 538-542.

38. Fuchs, M. S. and Kang, S.-H. 1981. Ecdysone and mosquito vitellogenesis: A critical appraisal. Insect Biochem., 11, 627-633.

39. Gordon, R. 1970. A neuroendocrine relationship between the nematode Hammerschmidtiella diesingi and its host Blatta orientalis. Parasitology, 61, 101-110.

40. Gwadz, R. W. and Spielman, A. 1973. Corpus allatum control of ovarian development in Aedes aegypti. J. Insect Physiol., 19, 1441-1448.

41. Gwadz, R. W. and Spielman, A. 1974. Development of the filarial nematode, Brugia pahangi in Aedes aegypti mosquitoes: Nondependence upon host hormones. J. Parasitol., 60, 134-137.

42. Hagedorn, H. H. 1974. The control of vitellogenesis in the mosquito, Aedes aegypti. Amer. Zool., 14, 1207-1217.

43. Hagedorn, H. H. and Fallon, A. M. 1973. Ovarian control of vitellogenin synthesis by the fat body in Aedes aegypti. Nature, Lond., 244, 103-105.

44. Hagedorn, H. H., Fallon, A. M. and Laufer, H. 1973. Vitellogenin synthesis by the fat body of the mosquito Aedes aegypti: Evidence for transcriptional control. Develop. Biol., 31, 285-294.

45. Hagedorn, H. H. and Judson, C. L. 1972. Purification and site of synthesis of Aedes aegypti yolk proteins. J. Exp. Zool., 182, 367-378.

46. Hagedorn, H. H., Kunkel, J. G. and Wheelock, G. 1978. The specificity of an antiserum against mosquito vitellogenin and its use in a radioimmunological precipitation assay for protein synthesis. J. Insect Physiol., 24, 481-489.

47. Hagedorn, H. H., O'Connor, J. D., Fuchs, M. S., Sage, B., Schlager, D. A. and Bohm, M. K. 1975. The ovary as a source of α-ecdysone in adult mosquitoes. Proc. Nat. Acad. Sci. USA, 72, 3255-3259.

48. Hagedorn, H. H., Shapiro, J. P. and Hanaoka, K. 1979. Ovarian ecdysone secretion is controlled by a brain hormone in an adult mosquito. Nature, Lond., 282, 92-94.

49. Harwood, R. F. and James, M. T. 1979. "Entomology in Human and Animal Health," Seventh Edition, Macmillan Co., New York.

50. Hazard, E. I., Andreadis, T. G., Joslyn, D. J. and Ellis, E. A. 1979. Meiosis and its implications in the life cycles of Amblyospora and Parathelohania (Microspora). J. Parasitol., 65, 117-122.

51. Hazard, E. I. and Chapman, H. C. 1977. Microsporidian pathogens of Culicidae (mosquitoes). In: Pathogens of Medically Important Anthropods (D. W. Roberts and M. A. Strand, eds.). Bull. W.H.O., 55, (Suppl. 1), 63-77.

52. Hazard, E. I. and Fukuda, T. 1974. Stempellia milleri sp. n. (Microsporida: Nosematidae) in the mosquito Culex pipiens quinquefasciatus Say. J. Protozool., 21, 497-504.

53. Hazard, E. I. and Oldacre, S. W. 1975. Revision of Microsporida (Protozoa) close to Thelohania, with descriptions of one new family, eight new genera and thirteen new species. U.S. Dept. Agric. Tech. Bull., 1530, 104 pp.

54. Hazard, E. I. and Weiser, J. 1968. Spores of Thelohania in adult female Anopheles: Development and transovarian transmission, and redescriptions of T. legeri Hesse and T. obesa Kudo. J. Protozool., 15, 817-823.

55. Henry, J. E. 1971. Experimental application of Nosema locustae for control of grasshoppers. J. Invertebr. Pathol., 18, 389-394.

56. Henry, J. E. 1981. Natural and applied control of insects by Protozoa. Annu. Rev. Entomol., 26, 49-73.

57. Hominick, W. M. and Davey, K. G. 1972. Reduced nutrition as the factor controlling the population of pinworms following endocrine gland removal in Periplaneta americana, L. Can. J. Zool., 50, 1421-1432.

58. Horobin, R. W. and Kevill-Davies, I. M. 1971. Basic fuchsin in acid alcohol: A simplified alternative to Schiff reagent. Stain Technol., 46, 53-58.

59. Humason, G. L. 1979. "Animal Tissue Techniques," Fourth edition, W. H. Freeman and Co., San Francisco.

60. Ilan, J., Ilan, J. and Ricklis, S. 1969. Inhibition of juvenile hormone of growth of Crithidia fasiculata in culture. Nature, Lond., 244, 179-180.

61. Jouvenaz, D. P. and Hazard, E. I. 1978. New family, genus, and species of Microsporida (Protozoa: Microsporida) from the tropical fire ant, Solenopsis geminata (Fabricus) (Insecta: Formicidae). J. Protozool., 25, 24-29.

62. Kellen, W. R., Chapman, H. C., Clark, T. B. and Lindegren, J. E. 1965. Host parasite relationships of some Thelohania from mosquitoes (Nosematidae: Microsporidia). J. Invertebr. Pathol., 7, 161-166.

63. Kellen, W. R., Chapman, H. C., Clark, T. B. and Lindegren, J. E. 1966. Transovarian transmission of some Thelohania (Nosematidae: Microsporidia) in mosquitoes of California and Louisiana. J. Invertebr. Pathol., 8, 355-359.

64. Kellen, W. R. and Lindegren, J. E. 1971. Modes of transmission of Nosema plodiae Kellen and Lindegren, a pathogen of Plodia interpunctella (Hubner). J. Stored. Prod. Res., 7, 31-34.

65. Kellen, W. R. and Lipa, J. J. 1960. Thelohania californica n. sp. a microsporidian parasite of Culex tarsalis Coquillett. J. Insect Pathol., 2, 1-12.

66. Kellen, W. R. and Wills, W. 1962. The transovarian transmission Thelohania californica Kellen and Lipa in Culex tarsalis Coquillett. J. Insect Pathol., 4, 321-326.

67. Kelly, T. J. and Fuchs, M. S. 1980. In vivo induction of ovarian development in decapitated Aedes atropalpus by physiological levels of 20-hydroxyecdysone. J. Exp. Zool., 213, 25-32.

68. Kelly, T. J., Fuchs, M. S. and Kang, S.-H. 1981. Induction of ovarian development in autogenous Aedes atropalpus by juvenile hormone and 20-hydroxyecdysone. Int. J. Invertebr. Reprod., 3, 101-112.

69. Kraminsky, G. P., Clark, W. C., Estelle, M. A., Geitz, R. D., Sage, B. A., O'Connor, J. D. and Hodgets, R. B. 1980. Induction of translatable mRNA for dopa decarboxylase in Drosophila: An early response to ecdysterone. Proc. Nat. Acad. Sci. USA, 77, 4175-4179.

70. Larson, J. R. and Bodenstein, D. 1959. The humoral control of egg maturation in the mosquito. J. Exp. Zool., 140, 343-377.

71. Laurence, B. R. 1977. Ovary development in mosquitoes: A review. Adv. Invertebr. Reprod., 1, 154-165.

72. Lawrence, P. O. 1982. Biosteres longicaudatus: Developmental dependence on host physiology. Exp. Parasitol. (In press).

73. Lea, A. O. 1963. Some relationships between environment, corpora allata, and egg maturation in aedine mosquitoes. J. Insect Physiol., 9, 793-809.

74. Lea, A. O. 1967. The medial neurosecretory cells and egg maturation in mosquitoes. J. Insect Physiol., 13, 419-429.

75. Lea, A. O. 1969. Egg maturation in mosquitoes not regulated by the corpora allata. J. Insect Physiol., 15, 537-541.

76. Lea, A. O. 1970. Endocrinology of egg maturation in autogenous and anautogenous Aedes taeniohynchus. J. Insect Physiol., 16, 1689-1696.

77. Lea, A. O. 1972. Regulation of egg maturation in the mosquito by the neurosecretory system: The role of the corpus cardiacum. Gen. Comp. Endocrinol., 3, 602-608.

78. Levine, N. D., Corliss, J. O., Cox, F.E.G., Deroux, G., Grain, J., Honigberg, B. M., Leedale, G. F., Loeblich, A. R., Lom, J., Lynn, D., Merinfeld, E. G., Page, F. C., Poljansky, G., Sprague, V., Vavra, J. and Wallace, F. G. 1980. A newly revised classification of the Protozoa. J. Protozool., 27, 37-58.

79. Lewis, R. R. and Knight, D. P. 1977. "Staining Methods for Sectioned Materials," North-Holland, New York.

80. Lillie, R. D. 1965. "Histopathologic Technic and Practical Histochemistry," Third edition. McGraw-Hill Co., New York.

81. Lord, J. C., Hall, D. W. and Ellis, E. A. 1981. Life cycle of a new species of Amblyospora (Microspora: Amplyosporidae) in the mosquito Aedes taeniorhynchus. J. Invertebr. Pathol., 37, 66-72.

82. Loubès, C., Maurand, J., Rousset-Galangau, M. and Grassé, P. 1976. Présence de complexes synaptonématiques dans le cycle biologique de Gurleya chironomi Loubès et Maurand, 1975: un argument en faveur d'une sexualité chez les microsporides? C.R. Hebd. Seances Acad. Sci. 282, 1025-1027.

83. Lumlinkhof, J., Lewis, L. C. and Berry, E. C. 1979. Effectiveness of integrating insecticides with Nosema pyrausta for suppressing populations of European corn borer. Environ. Entomol., 5, 143-146.

84. Maddox, J. V. 1966. Studies on a microsporidiosis of the armyworm Pseudaletia unipunctata (Haworth). Ph.D. Disservation, University of Illinois, Urbana, IL.

85. Maddox, J. V., Brooks, W. M. and Fuxa, J. R. 1981. Vairimorpha necatrix a pathogen of agricultural pests: Potential for pest control. In: "Microbial Control of Pests and Plant Diseases 1970-1980," (H. D. Burges, ed.) Academic Press, New York, pp. 587-594.

86. Maddox, J. V. and Sprenkel, R. K. 1978. Some enigmatic microsporidia of the genus Nosema. Misc. Publ. Entomol. Soc. Am., 11, 65-84.

87. Malavasi, A., DaCunha, A. B., Morgante, J. S. and Marques, J. 1976. Relationships between the gregarine Schneideria schneiderae and its host Trichosia pubescens (Diptera, Sciaridae). J. Invertebr. Pathol, 28, 363-371.

88. Marchoux, E. and Simond, P. L. 1906. Études sur la fièvre jaune. Deuxieme Memoire. Ann. Inst. Pasteur, Paris, 20, 16-40.

89. Maurand, J. and Loubès, C. 1973. Recherches cytochimiques sur quelques microspiridies. Bull. Soc. Zoo. France, 98, 373-378.

90. McLaughlin, R. E. 1971. Use of protozoans for microbial control of insects. In: "Microbial Control of Insects and Mites," (H. D. Burges and N. W. Hussey, eds.) Academic Press, New York, pp. 151-172.

91. Meola, R. and Lea. A. O. 1972. Humoral inhibition of egg development in mosquitoes. J. Med. Entomol., 9, 99-103.

92. Nadakal, A. M. and Nayar, K. K. 1968. Neural and hormonal influence on the fecundity and egg-laying of certain oxyurid nematodes inhabiting the hindgut of the cockroach Periplaneta american, L. Indian J. Exp. Biol., 6, 29-32.

93. Nayar, J. K. and Knight, J. W. 1981. Occurrence of ovariolar dilatations in nulliparous mosquitoes: Culex nigripalpus. Mosq. News, 41, 281-287.

94. Nenon, J. P. 1972a. Culture in vitro des embryos d'um Hyménoptère endoparasite polyembroynnaire: Ageniaspis fuscicollis (Encyrtus fuscicollus). Rôle des hormones de synthèse. C.R. Hebd. Seances Acad. Sci., 274, 3299-3302.

95. Nenon, J. P. 1972b. Culture in vitro des larves d'um Hyménoptère endoparasite polyembryonnaire: Ageniaspis fuscicollus. Rôle des hormones de synthèse. C.R. Hebd. Seances Acad. Sci., 274, 3409-3412.

96. Ohtaki, T. and Williams, C. M. 1970. Inactivation of α-ecdysone and cyasterone by larvae of the fleshfly, Sarcophaga peregrina and pupae of the silkworm Samia cynthia. Biol. Bull., 138, 326-333.

97. O'Meara, G. F. and Evans, D. F. 1977. Autogeny in saltmarsh mosquitoes induced by a substance from the male accessory gland. Nature, Lond., 267, 342-344.

98. Peracchia, C. and Mittler, B. S. 1972. Fixation by means of gluteraldehyde-hydrogen peroxide reaction products. J. Cell. Biol., 53, 234-238.

99. Pilley, B. M. 1976. A new genus Vairimorpha (Protozoa: Microsporida), for Nosema necatrix Kramer 1965: Pathogenicity and life cycle in Spodoptera exempta (Lepidoptera: Noctuidae). J. Invertebr. Pathol., 28, 177-183.

100. Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol., 17, 208-211.

101. Riddiford, L. M. 1975. Host hormones and insect parasites. In: "Invertebrate Immunity," (K. Maramorosch and R. E. Schope, eds.) Academic Press, New York, pp. 339-353.

102. Riddiford, L. M. and Truman, J. W. 1978. Biochemistry of insect hormones and insect growth regulators. In: "Biochemistry of Insects," (M. Rockstein, ed.) Academic Press, New York, pp. 307-357.

103. Roth, T. F. and Porter, K. R. 1964. Yolk uptake in the oocyte of the mosquito Aedes aegypti L. J. Cell Biol., 20, 313-332.

104. Rothschild, M. and Ford, B. 1964. Maturation and egglaying of the rabbit flea (Spillopsyllus cunicula Dale) induced by the external application of hydrocortisone. Nature, Lond., 223, 210-211.

105. Rozeboom, L. E. 1961. The effect of the gonadotropic hormone cycle of the adult mosquito on development of the malaria parasite. J. Parasitol., 47, 597-599.

106. Schlaeger, D. A. and Fuchs, M. S. 1974. Effect of dopa-decarboxylase inhibition on Aedes aegypti eggs: Evidence for sclerotization. J. Insect Physiol., 20, 349-357.

107. Schlaeger, D. A., Fuchs, M. S. and Kang, S.-H. 1974. Ecdysone-mediated stimulation of dopa decarboxylase activity and its relationship to ovarian development in Aedes aegypti. J. Cell Biol., 61, 454-463.

108. Schneider, F. 1951. Einige physiologische Benziehungen zwischen Syrphidenlarven und ihren Parasiten. Z. Angew. Entol., 33, 150-162.

109. Schoonhoven, L. M. 1962. Diapause and the physiology of host-parasite synchronization in Bupalus piniarius L. (Geometridae) and Eucarcelia rutilla Vill. (Tachinidae). Arch. Néerl. Zool., 15, 111-174.

110. Spielman, A., Gwadz, R. W. and Anderson, W. A. 1971. Ecdysone-initiated ovarian development in mosquitoes. J. Insect Physiol., 17, 1807-1814.

111. Spielman, A. and Wong, J. 1974. Dietary factors stimulating oogenesis in Aedes aegypti. Biol. Bull., 147, 433-442.

112. Sprague, V. 1977. Classification and phylogeny of the microsporidia. In: "Comparative Pathobiology," (L. A. Bulla and T. C. Cheng, eds.) Vol. 2, "Systematics of the Microsporidia," Plenum Press, New York, pp. 1-30.

113. Stein, G. 1956. Weitre Beiträge zur Biologie von Sphaerularia bombi Leon Dufour (1837). Z. Parasitenkd., 17, 383-393.

114. Stoffolano, J. G., Jr. 1967. The synchronization of the life cycle of diapausing face flies, Musca autumnalis, and of the nematode, Heterotylenchus autumnalis. J. Invertebr. Pathol., 9, 395-397.

115. Sutton, R. M., Gardiner, W. A., Kraus, D. W. and Noblet, R. 1979. Effects of ecdysone, juvenile hormone, and structurally-related compounds on Nomuraea rileyi, Beauveria bassiana, and Tritiarachium dependens in vitro. J. Ga. Entomol. Soc., 14, 364-373.

116. Thomson, H. M. 1958. Some aspects of the epidemiology of a microsporidian parasite of the spruce budworm, Choristoneura fumiferana (Clem.). Can. J. Zool., 36, 309-316.

117. Vavra, J. 1976. Structure of the microsporidia. In: "Comparative Pathobiology," (L. A. Bulla, Jr. and T. C. Cheng, eds.). Vol. 1, "Biology of the Microsporidia," Plenum Press, New York, pp. 87-109.

118. Vavra, J., Canning, E. V., Barker, R. J. and Desportes, I. 1981. Characters of microsporidian genera. (Workshop Proceedings, EMPO 3), Parasitology, 82, 131-142.

119. Weiser, J. 1976. Microsporidia in invertebrates: Host-parasite relations at the organismal level. In: "Comparative Pathobiology," (L. A. Bulla, Jr. and T. C. Cheng, eds.), Vol. 1, "Biology of the Microsporidia," pp. 163-201.

120. Weiser, J. 1977. Contribution to the classification of microsporidia. Vestn. Cesk. Spol. Zool., 24, 308-320.

121. Wilson, G. G. 1977. Observations of the incidence rates of Nosema fumiferanae in a spruce budworm, Choristoneura fumiferana population. Proc. Entomol. Soc. Ont., 108, 144-145.

122. Yoeli, M., Upmanis, R. S. and Most, H. 1962. Studies of filariasis. II. The relation between hormonal activities of the adult mosquito and the growth of Dirofilaria immitis. Exp. Parasitol., 12, 125-127.

123. Zimmack, H. L., Arbuthnot, K. D. and Brindley, T. A. 1954. Distribution of the European corn borer parasite Perezia pyraustae, and its effect on the host. J. Econ. Entomol., 47, 641-645.

124. Zimmack, H. L. and Brindley, T. A. 1957. The effect of the protozoan parasite Perezia pyraustae Paillot on the European corn borer. J. Econ. Entomol., 50, 637-640.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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